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ORIGINAL ARTICLE

Trophic range of *Heterobasidion annosum* (FR.) Bref. and *Phellinus pini* (Brot.) Bondartsev & Singer examined in laboratory conditions

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Abstract

In this paper the results of laboratory investigation on the range of wood decay caused by *Heterobasidion annosum* (Fr.) Bref. and *Phellinus pini* (Brot.) Bondartsev & Singer are described. Wood samples obtained from 25 different, both European and exotic tree species were used as the experimental substrate. The wood samples were exposed to mycelia of tested fungi for 30, 60 and 90 days, and subsequently the loss of their weight was assessed. The results showed a range of trophic preferences of *H. annosum* and *P. pini*. Better understanding of these processes could be useful in development of new methods for wood and forest protection against these extremely dangerous and common pests.

Key words

Heterobasidion annosum, Phellinus pini, white pocket rot, red ring rot, trophic preferences

Introduction

White pocket rot, also known as "red ring rot" or "white speck", is a specific type of wood decay. The fungi which cause of the decay are able to use all main wood components such as cellulose, hemicelluloses and lignin, however unevenly. As a result, a characteristic, spotted pattern appears on wood surface: numerous pockets filled with white cellulose are visible on darker, brown-red background. In the advanced decay stage, wood becomes brittle and breakable (Ważny 1968). Fom the economic perspective most important pathogens causing white pocket rot are: *Heterobasidion annosum* (Fr.) Bref. and *Phellinus pini* (Brot.) Bondartsev & Singer.

H. annosum is a parasitic or, less commonly, saprotrophic species widespread in Europe, North America and Asia (Ryvarden and Gilbertson 1993) and also recorded in Australia (Niemelä and Korhonen 1998). It has been recognised as one of the most serious parasites attacking trees of any age class. It causes mortality of young trees of various species both in cultivation and in young growth stands as well as is the reason of white pocket rot in the butt-end trunk of mature age class tree stands. In *Picea abies* (L.) H. Karst. and *Abies alba* Mill. the rot can achieve the height of around fifteen meters causing the ultimate depreciation of boles. An infected tree at first becomes red or purple red, and for some tree species (*P. abies*)

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– violet. Then it changes its colour into dark brown with well visible elongated pockets filled with white cellulose (Mańka 2005). In Poland, a range of occurrence and destructiveness of this fungus species have been widely researched (among others: Rykowski and Sierota 1983, Rykowski and Sierota 1984, Sierota 1996, Sierota 1997, Sierota 1998, Małecka and Sierota 2003, Sierota and Małecka 2003). *H. annosum* can also be found in conditions uncharacteristic for itself as a typical saprotroph decomposing timber. Among others, the fungus was recorded in England, on wood construction elements in mines where without access to sunlight it created fruit bodies of a shape unusual for itself (Gartwright and Findlay 1951).

Red ring rot is caused by a parasite commonly occurring in coniferous trees, mainly from the *Pinus* genus. It is characterised by wide geographic span as it inhabits Europe, northern part of Asia and the North American continent. In Poland, it is one of the most serious parasites of *Pinus sylvestris* (L.) – it is estimated that this fungus damages around 8% of pine timber forests (Mańka 2005, Szewczyk 2008). The fungus infects trees of the second age class or older. It causes an intensive and fast proceeding white pocket rot of hardwood only. The wood decay covers mostly the butt-end and the middle bole part, hence the parts of trees that are most valuable from the economic perspective. The infected tree changes its colour into dark red, and subsequently – in the advance stage of the decay, pockets filled with white cellulose appear on this background. This is caused by fungus nutritional strategy: in the initial phase of the decay it uses cellulose and lignin more or less evenly, but because wood contains twice as much of cellulose (Krzysik 1978) cellulose surplus is accumulated in spots creating characteristic white marks, noticeable with naked eye (Wiertelak 1933).

Because of the huge economic importance of *P. pini,* the fungus and the wood decay which it causes have been the objects of scientific research for last 120 years. The first author who investigated this topic was Hartig (1877). Detailed studies on biology of the pathogen were conducted by Percival (1933) In Poland, many discoveries in respect to diagnostics as well as damage prevention were reported by Zaleski and Wojtowicz (1937), Mańka and Chwaliński (1961) and Burkot-Klonowa (1974).

Such being the case, we can see that trophic preferences of *H. annosum* and *P. pini* have been well recognised and documented. On the other hand, however, not much has been known up to date about characteristics of these fungi observed in the laboratory conditions, and particularly when they infect a wider group of European and exotic tree species. The purpose of the presented study was research on *H. annosum* and *P. pini* in reference to their wood decay abilities in the conditions *in vitro*. The results can help to better understand biology of these fungi and to establish in the future more effective methods of control of these dangerous pathogens that cause huge economic losses in forests.

Materials and methods

The mycelia of *Heterobasidion annosum* (*sensu lato*) and *Phellinus pini* were obtained form forests within the area of the Radziwiłłów Forest Inspectorate (central part of Poland, ca. 80 km west of Warsaw). The mycelium of *H. annosum* was taken from wood originating from under the carpophore located on *Pinus sylvestris* trunk. The test material constituted wood of 25 tree species of which 16 were either domestic or introduced to Poland, and 9 were exotic – not found in Polish climatic conditions. The choice of tree species was influenced by the factors such as: frequency of occurrence, economic importance and presumed tree resistance to decomposition by fungi. The last criterion concerned mostly the chosen exotic species, that are believed to be highly resistant to decomposition and are often used in among others water and open air constructions – then in the conditions of high risk of decomposition. The list of tree species observed (with basic characteristics of their wood) is presented in Table 1. The samples of 50 mm x 25 mm x 15 mm size were taken out from the seasoned wood of each tree species. In the case of trees where sapwood and hardwood can be distinguished, the test wood samples were collected from the heartwood only. otherwise wood samples were obtained from the central part of tree trunk. All of the samples were measured using a calliper with the 0.1 mm accuracy and the volume of every individual sample was assessed. Then the samples were dried out to the state of complete dryness, initially at 60ºC and later at 105ºC with the use of an electric dryer. The time of drying was 72 hours

Table 1. The list of tree species used in the trial including main characteristics of their wood

Common name	Latin scientific name	Short name (see Fig. 1–3)	Wood density [g/cm^3]	Country of origin
European silver	*Abies alba* Mill.	A a	0.4395	Poland
Sycamore maple	*Acer pseudoplatanus* L.	A p	0.5401	Poland
European alder	*Alnus glutinosa* (L.) Gaertn.	A g	0.4897	Poland
Okoumé*	*Aucoumea klaineana* Pierre	A k	0.3957	Congo
White birch	*Betula pendula* Roth.	B p	0.5145	Poland
European hornbeam	*Carpinus betulus* L.	C b	0.7098	Poland
Iroko*	*Chlorophora excelsa* Benth. & Hook	C e	0.5241	Congo
Red beech	*Fagus sylvatica* L.	F s	0.6432	Poland
European ash	*Fraxinus excelsior* L.	F e	0.6269	Poland
Yatoba*	*Hymnaea* sp.	H s	0.9316	Brazil
Merbau*	*Intsia bakeri* Prain	I b	0.7158	Indonesia
European larch	*Larix decidua* Mill.	L d	0.5212	Poland
Wenge*	*Millettia laurentii* De Wild.	M l	0.7382	Congo
Badi*	*Nauclea trillesii* Merill	N t	0.7269	Congo
Norway spruce	*Picea abies* (L.) H. Karst.	P a	0.4295	Poland
Scots pine	*Pinus sylvestris* L.	P sy	0.3821	Poland
Common aspen	*Populus tremula* L.	P t	0.4495	Poland
Padouk*	*Pterocarpus soyauxii* Taubert	P so	0.6405	Congo
English oak	*Quercus robur* L.	Q ro	0.5806	Poland
Northern red oak	*Quercus rubra* L.	Q Ru	0.6425	Poland
Crack willow	*Salix fragilis* L.	S f	0.4478	Poland
Ipe*	*Tabebuja* sp.	T sp	0.9511	Brazil
Small-leaved lime	*Tilia cordata* Mill.	T c	0.4424	Poland
Samba*	*Triplochiton scleroxylon* K. Schum.	T sc	0.3377	Cameroon
Field elm	*Ulmus carpinifolia* Gleditsch	U c	0.5604	Poland

* names commonly used in timber industry

minimum. Directly after removing from the dryer, the samples, were weighed with the use of a scale with 0.001 g accuracy. The density of each wood sample was assessed. The samples chosen for the tests were the ones that demonstrated similar value of density.

Into each of glass containers of 1500 ml capacity, sterilized with the use of autoclave (at 121ºC during min. 30 minutes), 20 ml of agar-maltose-wort medium was poured. The composition of the medium was: Difco agar – 20g, Difco maltose extract – 15 g, distilled water – 750 ml, non-hopped beer wort 250 ml. The beer wort used in the experiments came from 'Jabłonowo' Brewery and was obtained once from same container so as to keep the medium. standardized. After 24 hours the mycelia inoculates of *H. annosum* and *P. pini* were introduced onto the solidified medium. Then closed containers were put into the incubator at the temperature 21ºC. After 14 days, two wood samples sterilised earlier with the use of the radiation method were put on glass pads onto the grown mycelium in each of the containers. The radiation sterilisation of the wood samples was performed in the Institute of Nuclear Chemistry and Technology in Warsaw. The use of glass pads protected the wood samples from humidity permeating from the medium so as to avoid distorting of the results. Then the containers were put back in the incubator and

separate batches were removed after 30, 60 and 90 days. For each of the incubation periods there were 6 samples (3 containers) of wood of each of tested tree species. After removing from the containers the samples were cleansed of mycelia remains and dried again in the incubator and afterwards weighed with the use of the scale with 0.001 g accuracy. The loss of sample weight when compared to the results of the first weighing reflected a degree of decomposition of each individual sample by the fungi. This was expressed as the percent value according to the following formula:

$$\Delta = [(G_0 - G_1) / G_0] \times 100\ (\%)$$

where:
Δ – percentage of loss of sample weight
G_0 – sample weight (g) prior to incubation
G_1 – sample weight (g) after incubation.

In total 900 samples of wood placed in 450 containers were studied. The differences of wood weight loss among 25 species of trees were assessed using analysis of variance and multiple comparison test (LSD method – Least Significant Differences). Separate statistical analyses were conducted for the 30 -, 60 – and 90 – day periods of decomposition at the 95% confidence level.

Results

The results concerning wood decay caused by examined fungi in investigated tree species after 30, 60 and 90 days of the exposure to mycelium are presented in Table 2. The obtained values proved the existence of differences in the loss of dry weight of wood samples of observed tree species. Statistical significance of these differences is illustrated on Figures 1–3. The results obtained from the trials conducted on mycelia of tested individual fungi species are as follows:

Heterobasidion annosum: after 30 days of exposure to the mycelium, the average loss of weight of wood samples of all observed species amounted to 1.83%. Most decomposed were the wood samples of *Acer pseudoplatanus* L. (6.29%), *Populus tremula* L. (5.44%) and *Carpinus betulus* L. (4.68%), and least decomposed were the samples of *Intsia bakeri* Prain (0.01%), *Hymnaea sp.* (0.02%) and *Pterocarpus*

Table 2. Average percentage weight loss of wood samples of tested tree species after 30, 60 and 90 days exposure to the mycelia of tested fungi

Tree species	*Heterobasidion annosum*			*Phellinus pini*		
	30 days	60 days	90 days	30 days	60 days	90 days
Abies alba	2.50	5.64	12.71	0.89	1.68	2.58
Acer pseudoplatanus	6.29	12.13	13.18	0.57	0.95	1.34
Alnus glutinosa	1.97	19.33	23.20	0.47	1.21	1.97
Aucoumea klaineana	0.78	3.97	7.43	0.19	0.21	0.36
Betula pendula	4.27	14.16	14.57	0.28	0.41	0.49
Carpinus betulus	4.68	10.73	13.45	0.47	0.61	0.99
Chlorophora excelsa	0.18	0.89	0.91	0.02	0.03	0.05
Fagus sylvatica	0.52	10.52	10.96	0.10	0.34	0.51
Fraxinus excelsior	1.34	6.13	8.37	0.04	0.09	0.14
Hymnaea sp.	0.02	0.51	0.64	0.06	0.07	0.08
Intsia bakeri	0.01	0.03	0.06	0.03	0.04	0.06
Larix decidua	1.37	7.44	8.39	0.27	0.68	1.68
Millettia laurentii	0.10	0.20	0.31	0.13	0.17	0.19
Nauclea trillesii	0.05	1.43	1.66	0.33	0.42	0.54
Picea abies	4.39	9.85	11.42	0.29	1.01	1.28
Pinus sylvestris	1.50	4.11	5.41	0.08	0.87	1.37
Populus tremula	5.44	15.00	15.33	0.43	0.87	1.28
Pterocarpus sayauxii	0.03	0.07	0.09	0.08	0.15	0.24
Quercus robur	0.06	0.78	0.87	0.17	0.28	0.41
Quercus rubra	0.39	5.44	6.97	0.22	0.67	1.18
Salix fragilis	3.32	6.19	8.28	0.17	0.41	0.53
Tabebuja spp.	1.33	1.46	1.54	0.02	0.08	0.12
Tilia cordata	2.58	2.96	18.91	0.42	0.89	1.01
Triplochiton scleroxylon	0.12	1.91	2.94	0.79	1.24	1.89
Ulmus carpinifolia	2.49	9.92	10.80	0.12	0.47	0.81

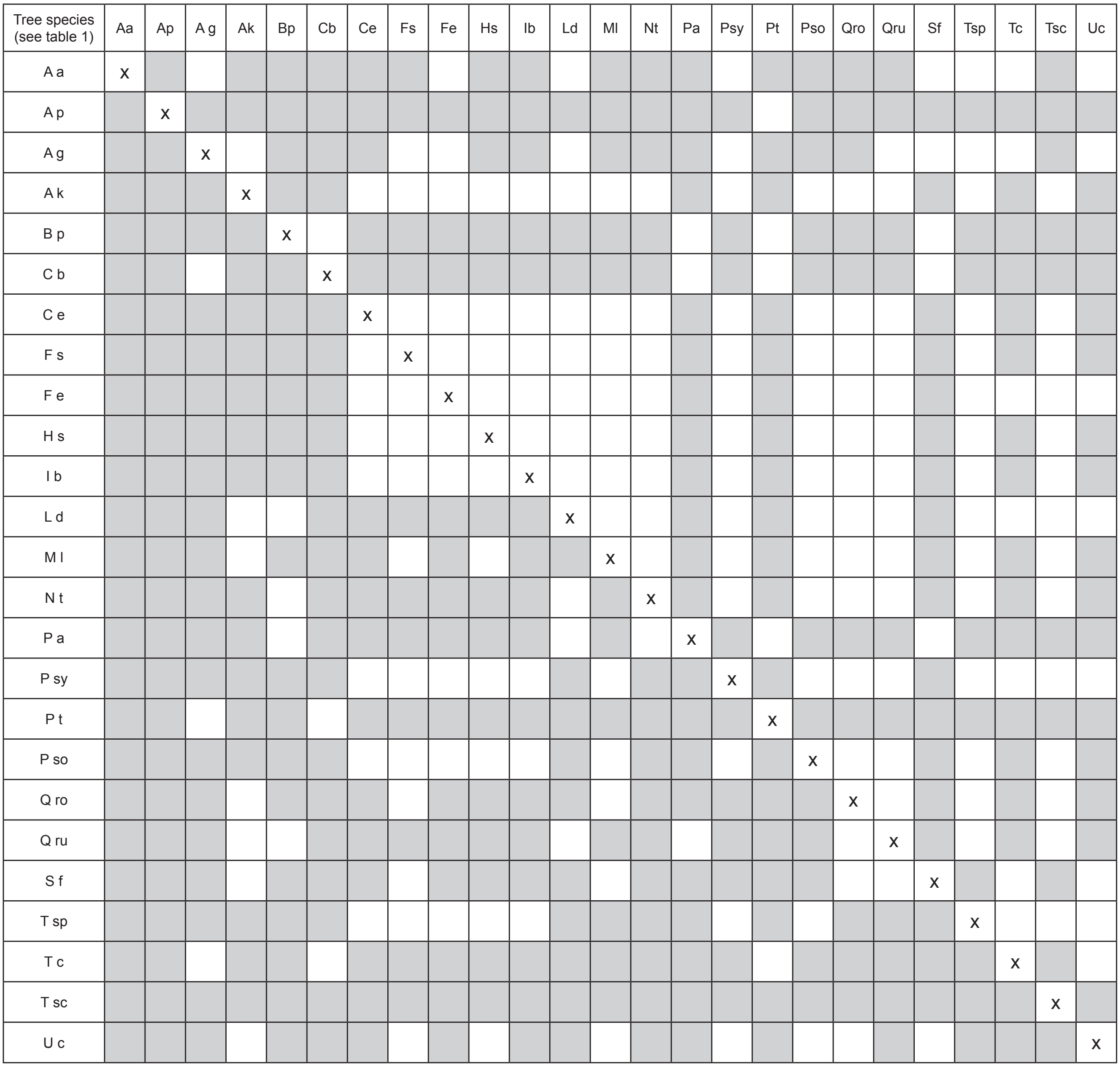

Figure 1. The variance of the wood decay range observed in 25 tree species exposed to *Heterobasidion annosum* and *Phellinus pini* for 30 days (dark color indicates statistically significant differences – by LSD tests at the 95% confidence level)

soyauxii Taubert (0.03%), After 60 days of exposure to the mycelium the average loss of weight of wood samples of all tested tree species amounted to 6.03%, Most decomposed were the wood samples of *Alnus glutinosa* (L.) Gaertn. (19.33%), *P. tremula* (15.00%) and *Betula pendula* Roth. (14.16%), and least decomposed were the samples of *I. bakeri* (0.03%), *P. soyauxii* (0.07%) and *Milletia laurentii* De Wild. (0.20%). After 90 days of exposure to the mycelium,the average loss of weight of wood samples of all the species amounted to 7.94%. Most decomposed were the wood samples of *A. glutinosa* (23.20%), *Tilia cordata* Mill. (18.91%) and *P. tremula* (15.33%), and least decomposed ones were *I. bakeri* (0.06%), *P. soyauxii* (0.09%) and *M. laurentii* (0.31%),

Phellinus pini: after 30 days of exposure to the mycelium the average loss of weight of wood samples of all the species amounted to 0.27%. Most de-

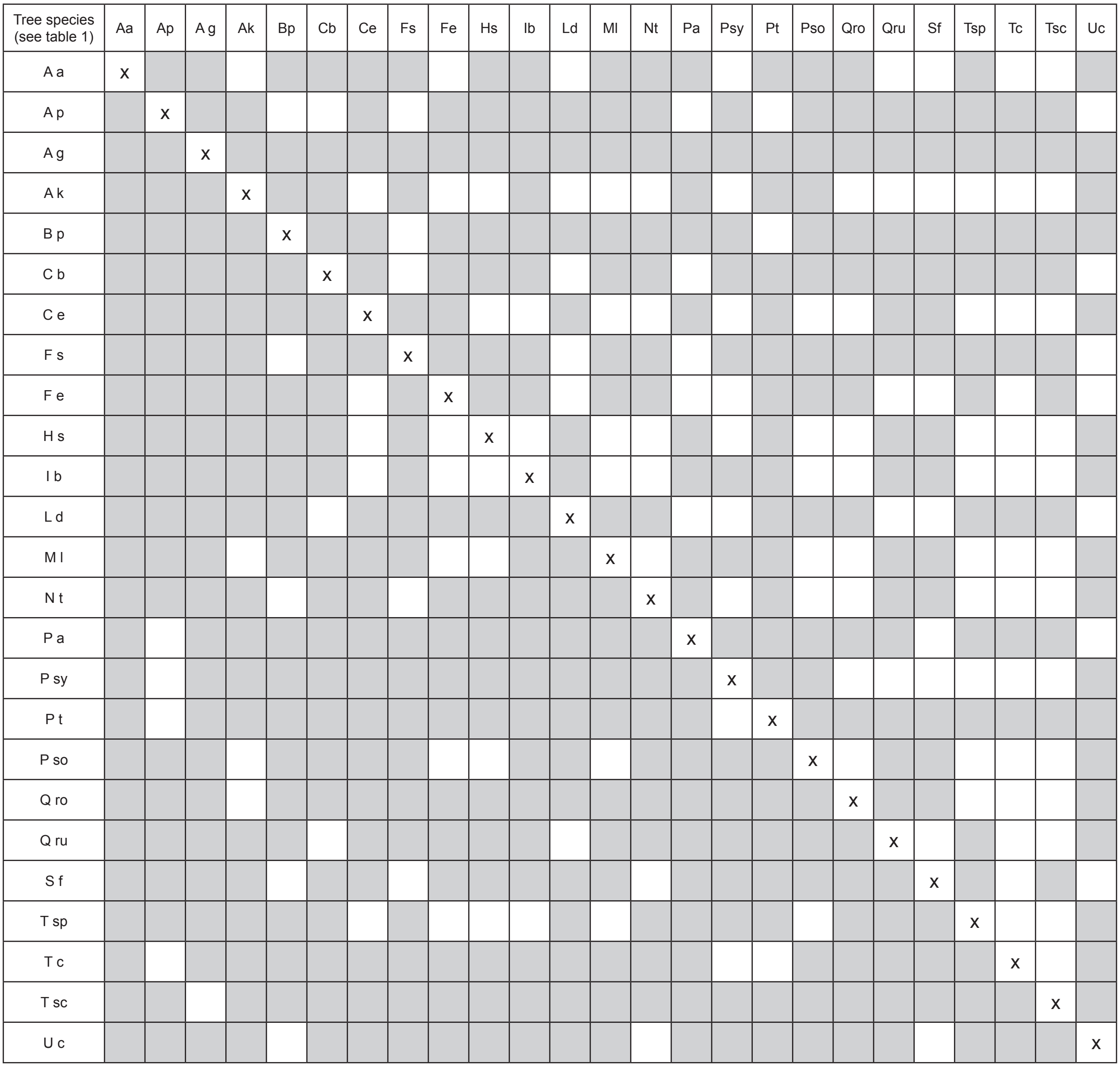

Figure 2. The variance of the wood decay range observed in 25 tree species exposed to *Heterobasidion annosum* and *Phellinus pini* for 60 days (dark color indicates statistically significant differences – by LSD tests at the 95% confidence level)

composed were the wood samples of *Abies alba* (0.89%), *Triplochiton scleroxylon* K. Schum. (0.79%) and *A. pseudoplatanus* (0.57%), and least decomposed ones were *Chlorophora excelsa* Benth. & Hook (0.02%), *Tabebuja sp.* (0.02%) and *I. bakeri* (0.03%), After 60 days of exposure to the mycelium, the average loss of weight of wood samples of all the species amounted to 0.55%,. Most decomposed were the wood samples of *A. alba* (1.68%), *T. scleroxylon* (1.24%) and *A. glutinosa* (1.21%), and least decomposed ones were *Chlorophora excelsa* (0.03%), *Intsia bakeri* (0.04%) and *Hymnaea sp.* (0.07%), After 90 days of exposure to the mycelium, the average loss of weight of wood samples of all the species amounted to 0.84%, Most decomposed were the wood samples of *A. alba* (2.58%), *A. glutinosa* (1.97%) and *T. scleroxylon* (1.89%), and least decomposed ones were *Ch. excelsa* (0.05%), *I. bakeri* (0.06%) and *Hymnaea sp.* (0.08%).

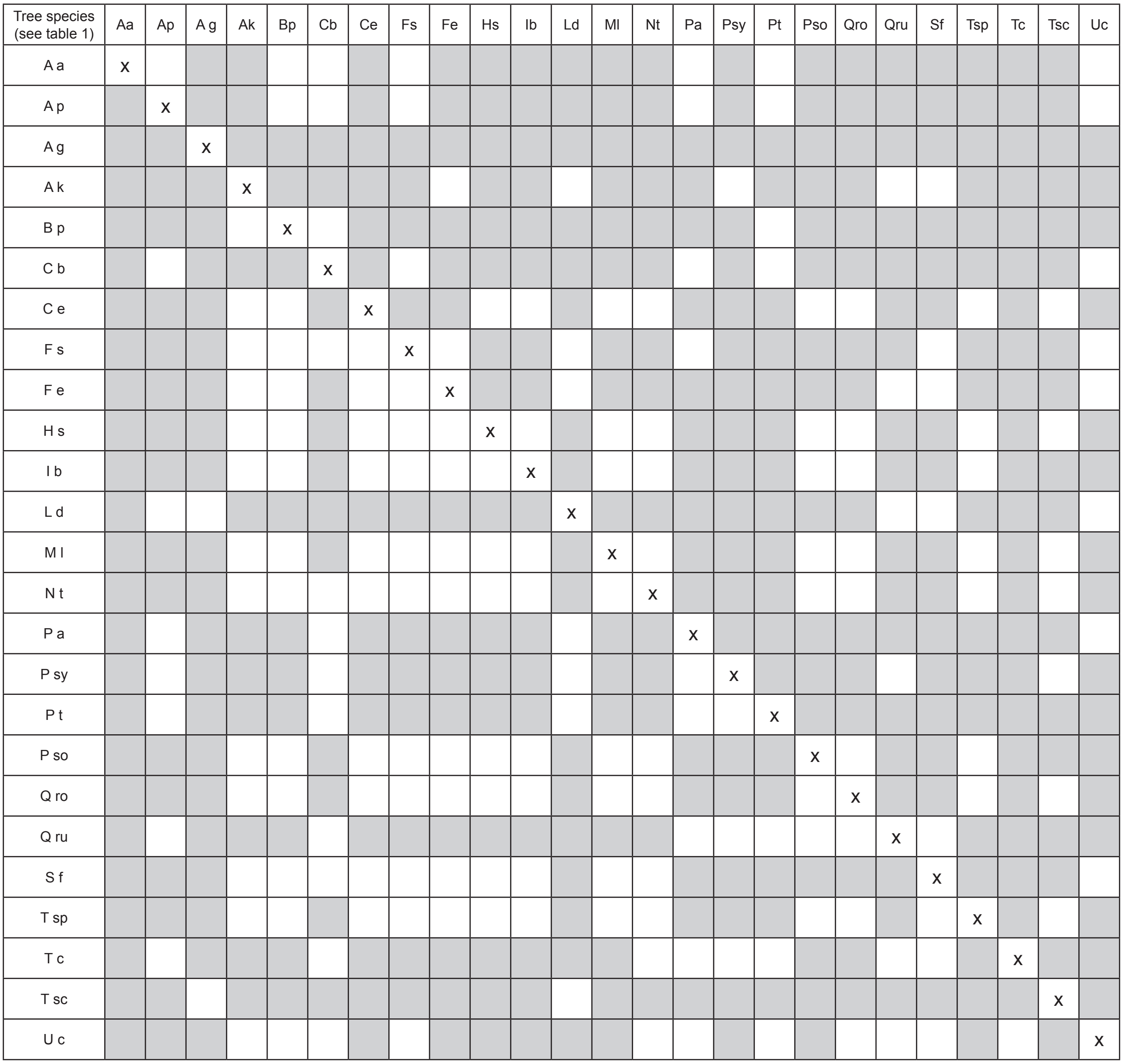

Figure 3. The variance of the wood decay range in 25 tree species exposed to *Heterobasidion annosum* and *Phellinus pini* for 90 days (dark color indicates statistically significant differences – by LSD tests at the 95% confidence level)

Discussion

Basing on the results it can be stated, that the range of trophic abilities and preferences of *Heterobasidion annosum* and *Phellinus pini* isolates under conditions *in vitro* does not fully correspond to the abovementioned characteristics of these species observed in nature.

As Ryvarden and Gilbertson (1993) state, *H. annosum* (*sensu lato*) in natural conditions occur mainly on living or dead coniferous trees of the genera *Abies*, *Picea* and *Pinus* and less often on *Larix* and *Juniperus*. The fungus also occurs on broadleaved trees and bushes, among others of the genera: *Acer*, *Alnus*, *Betula*, *Carpinus*, *Corylus*, *Cratageus*, *Fagus*, *Fraxinus*, *Lonicera*, *Prunus*, *Pyrus*, *Robinia*, *Sambucus*, *Salix*, *Sor-*

bus and *Ulmus*, and – sporadically – even on shrubs of the *Ericaceae* family such as *Calluna sp.*, *Empetrum sp.* and *Vaccinium sp.* According to Kotlaba (1984), on the area of Central Europe, the fungus definitely prefers coniferous trees as its host, and most of all *Picea abies* (68% of cases), various species of the genus *Pinus* (17%) and *Abies alba* (8%). As other hosts the author enumerates among others: *Abies concolor* (Gordon and Glend.) Lindl. ex Hildebr., *A. homolepis* Siebold and Zucc., *Larix decidua* Mill., *Acer pseudoplatanus*, *Alnus glutinosa*, *Carpinus betulus*, *Catalpa sp.*, *Populus tremula*, *Prunus avium* (L.) L., *Prunus laurocerasus* L., *Prunus spinosa* L., *Pyrus communis* L., *Sambucus nigra* L., *S. caprea* L., *Sorbus aucuparia* L. and *Tilia cordata*.

Laboratory tests of wood decomposition by *H. annosum* isolate proved that the fungus caused very intense decay of wood originating from many tree species. Fastest wood decomposition was observed in *A. glutinosa* (23.20% of dry weight loss after 90 days of exposure to the mycelium), *T. cordata* (18.91%) and *Populus tremula* (15.33%), and these are broadleaved species. In the case of coniferous species, fastest wood decomposition was observed in *A. alba* (12.71%), *P. abies* (11.42%), *L. decidua* (8.39%), and right next to these, there was positioned *Pinus sylvestris* (5.42%), from which the mycelium that was used in this study was obtained. The species that proved to be completely resistant to decomposition by *H. annosum* isolate tested in laboratory conditions were *Intsia bakeri* and *Pterocarpus soyauxii* in the case of which no losses of dry weight of samples were observed after 90 days of exposure to the mycelium.

It can be stated that *H. annosum* tested in laboratory conditions behaves as a typical saprotrophic-polyphagous species, however its range of trophic preferences is slightly different than that observed in nature, where the fungus occurs mainly as the parasite. That phenomenon can be explained by the fact that wood outside of a living tree organism (i.e. dead) maintains different chemical properties, namely it lacks some of metabolic products which are most likely to have significant influence on the range of trophic abilities of wood decomposing fungi, and – at the same time – on the range of wood resistance to depreciation by this group of organisms.

P. pini is the species which in natural European conditions occurs almost only on trees of the genus *Pinus* (Ryvarden and Gilbertson 1994), however Kotlaba (1984) mentions three cases of the occurrence of this fungus observed on *L. decidua* within the territory of former Czechoslovakia. In Asian countries as well as in Canada and the USA it also occurs on other coniferous trees of the genera *Abies*, *Larix*, *Picea* and *Pseudotsuga* (Mańka 2005).

In vitro tests of wood decomposition proved that tested *P. pini* isolate does not cause very intensive depreciation of wood material in laboratory conditions (that is outside of tree stands). In accordance with natural preferences of this fungus species, wood of coniferous tree wood originating from *A. alba* and *L. decidua*. Similar quantities of decomposition were also observed for a variety of European broadleaved species, among others *A. glutinosa*, *Acer pseudoplatanus*, *Populus tremula* and *Quercus rubra* L., as well as for exotic tree species (*Triplochiton scleroxylon*). Nevertheless, the average pace of wood decomposition for all of the tested tree species in general was relatively low (0.84% of dry weight loss after 90 days of exposure to the mycelium). Therefore, it can be stated that dead wood tested in conditions *in vitro* did not provide a good base for decomposition by *P. pini*. This phenomenon can be explained by the fact, that this species does not occur on dead wood in nature being the typical parasite bound to living trees.

Conclusions

– Under *in vitro* conditions the isolate of *Heterobasidion annosum* decomposed the fastest broadleaved wood originating from *Alnus glutinosa*, *Tilia cordata* and *Populus tremula*. In the case of coniferous species the wood of *Abies alba* and *Picea abies* was decomposed the fastest
– Under *in vitro* conditions the isolate of *Phellinus pini* does not cause severe wood decomposition. Wood decomposed the fastest originated from coniferous species: *Abies alba* and *Larix deciduas*. Similar degree of decomposition was observed for broadleaved tree species, among others *Alnus glutinosa*, *Acer pseudoplatanus*, *Populus tremula* and *Quercus rubra*
– The range of abilities and reference of the tested fungi towards wood of various European and exotic tree species was different than that observed in nature. This can be explained by characteristics of dead wood, i.e. location outside of the living tree organ-

ism leading to wood deprivation of a part of chemical compounds which naturally occur in living trees.

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